ANDROLOGY

The Ratio of X- and Y-Bearing Sperm in Ejaculates of Men with Three or More Children of the Same Sex

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Purpose: *The present study evaluated the proportions of X-bearing and Y-bearing sperm within the semen of donors who were the declared fathers of three or more sons or daughters.*
Methods: *The proportions of sperm were determined using dual-color fluorescence in situ hybridization to identify the X and Y chromosomes.*
Results: *The only difference observed was in semen volume. There was no increase in the proportion of Y-bearing sperm for men with only sons (49.7 ± 1.3%) or of X-bearing sperm for men with only daughters (44.8 ± 2.6%).*
Conclusions: *A preponderance of either sons or daughters in a family cannot be explained simply by an altered ratio of X-bearing and Y-bearing sperm in the father's semen.*

KEY WORDS: fluorescence in situ hybridization; sperm; X chromosome; Y chromosome.

INTRODUCTION

The ratio of human males to females appears to be subject to many influences, including behavioral and psychological factors (1) and the environment (2,3). However, the mechanisms are unclear and three possible reasons are (i) an alteration in the ratio of X- and Y-bearing sperm, (ii) selection of sperm within the female reproductive tract, and (iii) differential implantation and survival rates of embryos. Previous attempts have been made to assess the ratio of X- and Y-sperm in either sperm donors or men with all daughters. However, there are significant problems with the older methods of sperm assessment, and the earlier reports show conflicting evidence with various reports showing an excess of either X-sperm (4–7) or Y-sperm (8). More recent reports have shown no difference in the sperm ratio (9–11).

The present study re-examined the ratio of X- and Y-bearing sperm in semen. Men with three or more children of the same sex, and thus an apparent altered sex ratio in their family, were investigated using dual-color fluorescence in situ hybridization (FISH) specific for both the X and the Y chromosomes.

MATERIALS AND METHODS

Subjects were recruited following an article in the local newspaper appealing for men with three or more children of the same sex, and the project was approved by the Institutional Ethics Committee. Semen samples were produced by masturbation, with no restriction on the period of prior abstinence, and analyzed in accordance with standard World Health Organization procedures (12). The samples were prepared for FISH by washing an aliquot twice with phosphate-buffered saline (PBS) before being fixed in a 3:1 methanol:acetic acid solution. The fixed sperm suspensions were dropped into precleaned slides and then heated at 60°C for 15 min. The sperm heads were decondensed using a drop of 3 *M* sodium hydroxide at 22°C for approxi-

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mately 3 min. The slides were dehydrated using sequential solutions of 70, 95, and 100% ice-cold ethanol solutions.

The probes DXZ1 and DYZ1 (Oncor, Gaithersburg, MD) were specific for α satellite DNA in the centromere region of the X chromosome and satellite 3 DNA at Yq12, respectively. The slides containing the sperm were incubated with the probes for 18 hr at 37°C, in a humidified environment. The DXZ1 (X-chromosome probe) was detected using α-DIG rhodamine, while DYZ1 (Y-chromsome probe) was detected using AV-FITC and amplified using BIOT-αAV. The sperm heads were stained with DAPI. The slides were examined using a Leitz Laborlux Ploemopak fluorescence microscope with Leica filters N2 for the rhodamine signal, I3 for fluorescein isothiocyanate, and A for DAPI.

For each sample a minimum of 400 sperm heads was counted. This is the minimum number of sperm required to identify a statistically significant change in the sex ratio (13).

Group data are expressed as means and standard errors of the mean and compared using Student's t test. Differences were considered significant at $P < 0.05$.

RESULTS

As shown in Table I, the only differences observed were in semen volume, concentration of sperm, and total number of sperm per ejaculate. No increase in the proportion of Y-bearing sperm for men with only sons, or in X-bearing sperm for men with only daughters, was detected.

DISCUSSION

The identification and counting of the sex chromosomes within sperm have been fraught with problems.

Table I. Characteristics of the First Semen Sample Produced by Men Having All Sons (n = 12) or All Daughters (n = 7)

Mean parameter	Children	
	≥3 sons	≥3 daughters
Semen volume (ml)	4.1 (0.4)[a]	2.7 (0.1)*
Sperm concentration ($\times 10^6$/ml)	73.0 (7.2)	121.4 (8.5)
Sperm per ejaculate ($\times 10^6$)	296.9 (37.4)	326.5 (27.5)
Proportion of Y-bearing sperm	49.7% (1.3%)	52.7% (2.7%)
Proportion of X-bearing sperm	48.6% (1.3%)	44.8% (2.6%)
Proportion of unstained sperm	1.7% (0.1%)	2.5% (0.5%)

[a] Standard error of the mean in parentheses.
* $P < 0.02$.

Previous methods have had a number of major limitations, for example, the following.

(a) Staining of sperm with quinicrine hydrochloride gives a brightly fluorescent dot where it binds to the heterochromatic region on the Y chromosome. Unfortunately, counting can be confounded by non-Y signals from other heterochromatic regions (e.g., the 9-chromosome heterochromatin) or a small heterochromatic region of the Y chromosome, which can make the Y chromosome difficult to distinguish. Counting of X-bearing sperm relies on the absence of a fluorescent spot and is related to any problems in counting the Y-bearing sperm.

(b) The use of hamster oocytes relies on penetration by human sperm, formation of pronuclei, and decondensation of the chromosomes. The possibility of a preferential selection of sperm based on their functional properties cannot be excluded, and oocytes with more than one sperm inside can be difficult to analyze.

Only two studies have examined sperm in the semen of men with children of the same sex, and in each case they recruited men with daughters only. Both used quinicrine staining and reported reduced proportions of sperm with a Y chromosome (4,6). Other reports have examined sperm from donors with no evidence of an altered ratio of children and, using quinicrine staining, found either an increase in the proportion of sperm with a Y chromosome (8) or no difference (9). Investigations using hamster oocytes report an excess of sperm with an X chromosome (5,14). More recently, studies based on FISH found no difference in the sperm ratio in the ejaculates of donors (10,11) prior to the evaluation of methods for the preferential selection of either X-or Y-bearing sperm for sex preselection. The present study has therefore combined the positive elements of the above studies, namely, FISH technology and the selection of men with children all of the same sex.

The only difference found in men who were the stated fathers of either boys only or girls only was the semen volume. However, as no requirements for sexual abstinence were imposed on the subjects before collection of the test sample, variations in the frequency of preceding sexual activity cannot be excluded as a causative factor.

In summary, the present study has found no evidence for an altered ratio of X- and Y-bearing sperm in the semen of men with three or more children all of the same sex. If the occurrence of such families is more than a chance happening, then the possibility of selection of sperm during the fertilization process (15) or

differential implantation/survival of the embryos must be considered.

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